TRANSLATION NO. 41

DATE: July 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Russian Copy 1
Source - Dr. Foley
#141

TULAREMIA (1944-1946)

"On the basis of data obtained by study of the immunity to tularemia, Gayskiy recently (1944) proposed for the vaccination of man two diluted strains, Ondatra IV and No. 15.

"...tularemia vaccine obtained from living attenuated vaccine microbes... encounters serious obstacles as a result of its instability when stored; in but a few hours of storage at room temperature, or in 12-15 days of storage at refrigerator temperature, this vaccine loses its effectiveness...Thus it became urgently necessary to develop a highly effective vaccine preparation from living cells capable of preserving its immunogenic properties for an extended time.

"To develop a preparation capable of satisfying the demands indicated, we utilized the above described strains of Ondatra IV and No. 15 obtained from Gayskiy. We used the method of drying the frozen suspension of microbes of dry vaccine from the indicated strains in a special medium in a high vacuum.

"...under conditions of drying microbe cultures in appropriate media (Drying media), this process of drying, suggested by Shekkel in 1909, permits the production of dry preparations capable of retaining their properties unchanged for a long time. Media best adapted for this purpose proved to be those containing saccharose or other carbohydrates (maltose and lactose) in amounts of 10-18%, with an added 1.25-1.5% of gelatin for the purpose of improving drying conditions.. Our experiments have shown that the best media for drying living tularemia vaccine were saccharose-agar-gelatin (saccharose 10-35%, agar 0.1%, gelatin 1.25-0.5%) and hepatic saccharose-agar-gelatin 1.25-0.5%) and hepatic saccharose-agar-gelatin (the latter serves concurrently as a growth culture).

"Drying of living tularemia vaccine was carried out in an apparatus consisting of diverse equipment developed forvaccine drying be fellows of MIIEG... and of a high vacuum pump of the Stokes Firm (model 412 and 212) that permits the evaporation of water vapor into the atmosphere without the use of chemical absorbants or low temperature for humidity condensation.

"The intricacy of preparing the media known at the present time (egg yolk... and cystins-blood agar...) for growing causal agents of tularemia and the impossibility of their use in mass production of bacterial preparations has compelled many authors to search for simpler media...our own numerous investigations enabled us to propose the following media for mass production of tularemia bacteria preparations: (1 semi-liquid agar consisting of pancreatin hydrolysates of liver and gelatin, saccharose, white gelatin, starch, agar and water; (2) a thick agar medium in two variants, the first of which contains pancreatin hydrolysates of liver, blood and gelatin, milk serum, cystine, starch, table salt, agar and water, and the second--pancreatin hydrolysates of blood, autolycates of yeasts, milk serum, cystine, starch, table salt, glucose, agar and water,

"For the purpose of preserving in the media the largest possible amount of vitamins (growth factors) needed for the cultivation of the causal agent of tularemia, the hydrolysates of liver and blood, contrary to the generally accepted method, were prepared without a preliminary thermal treatment, i.e., from raw material. For the precipitation of yolks and other substances which retard microbe growth, all hydrolysates were treated with aluminum sulfate. A slightly alkaline reaction (pH 7.1-7.2) was established in the growth media, and in the drying media, a neutral reaction.

"The proposed media are sterilized in an autoclave at a temperature of 120°C. If specific rules are observed in dispensing, then those media produce a high yield of microbes (on the average, on 1 cc ofmedium no less than 1.5 billion

from a semi-liquid, and 3 billion from a thick agar one): as regards their properties, tularemia cultures grown on them do not differ from the cultures obtained from egg yolk media.

"The media indicated were used for the preparation of dry living vaccine of two variants; from cultures on a semi-liquid medium (containing 4% saccharose instead of salt) and from cultures derived from a thick agar medium. In order to obtain dry vaccine of the first variant, an additional solution of saccharose up to 10% was added to a 2-3 full-day old culture on semi-liquid medium, after which it was stirred and poured in ampules at 1cc each and then dried. As a result, the semi-liquid medium was simultaneously a medium for growth and a medium for drying. At the beginning this semi-liquid medium was prepared without saccharose, but with an addition of 0.85% table salt. It proved subsequently that dry vaccine prepared from cultures grown on such a medium contained but a small amount of living microbes and quickly lost its effectiveness when stored. Therefore, saccharose (4%) was substituted for table salt. To prepare dry vaccine of the second variant, the cultures on a thick agar medium following 2-3 full-days of growth at 37°C, were washed with a drying medium, examined as to purity, diluted with the same drying medium up to a titre of 2-5 billion microbes on 1 cc and were poured in ampules at 1 cc each. Before drying, the vaccine poured into ampules was frozen in ice mixed with table salt. After their drying for a period of 14-18 hours, the ampules were disconnected so as to preserve high vacuum within them.

"The investigations conducted showed that the drying of cultures of vaccine strains, suspended in media containing 10-15% saccharose, 0.1% agar and 1.3% gelatin, permits obtaining a dry preparation with a high content of living microbes capable of enduring prolonged storage.

"It must be Pointed out that drying cultures of vaccine in a saccharose-agar-gelatin or in a saccharose-gelatin medium produced in both cases dry vaccine containing an almost equal number of living microbes. The saccharose-gelatin dry vaccine, however, proved less stable during storage...Drying suspensions of living vaccine in other media without saccharose and agar led to intensified dying-off of living microbes in the process of drying as well as in storage in dry form even at refrigerator temperature."

"The dry living tularemia vaccine...remains immunogenic for a long time. Thus, the dry vaccine, kept at 18 C. for 270 days, at 26 C. for 75 days, or at 2-4 C. for 1.5 years, after a single subcutaneous vaccination in doses from 10 to 25 million microbes, protects 95-100% of guinea pigs and white mice from subsequent subcutaneous infection with 1,000-2,000 fatal doses of the virulent culture.

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"The study of immunogenic properties of the dry living vaccine...confirmed once again that the No. 15 strain, in comparison with the Ondatra IV strain, has more marked immunogenic properties.

"The study of the dry living tularemia vaccine on man-initially on 52 volunteers who received the vaccine in doses ranging from 7.5 to 250 million microbes--and then on more than 30,000 men who received the vaccine in doses ranging from 12.5 to 50 million...has shown that all the above described variants of the vaccine are endured by man relatively easily, without a prolonged reaction of complications. To the subcutaneous introduction of even large doses of the

vaccine (from 100 to 250 million microbes), the men reacted with a brief local and general reaction which ran benignly. Moreover, reaction on the part of the regional glands was not observed in all cases. In doses of up to 50 million, the vaccine is endured by man entirely easily, and it causes mainly a mild local reaction and in rare cases, a weak reaction of part of the regional lymphatic glands, and still less frequently, a temperature reaction.

"Persons who have had tularemia react to the introduction of the living vaccine considerably more strongly, which must be explained by the presence of increased sensitivity in them.

"However, doses which do not exceed 25 million microbes do not cause--- even in those who have had the illness---a sharply pronounced reaction which disrupts their work capacity.

"Results of the study of the vaccine from different strains on men, as in experiment, have shown that the vaccine from the No. 15 strain, in comparison with the vaccine from the Ondatra IV Strain, has more pronounced allergenic and antiganic properties; even after a month it causes in all inoculated men a positive agglutination reaction and an allergy condition, whereas the vaccine from the Ondatra IV strain, one month after the inoculation, causes an agglutination reaction in only 35%, and an allergy reaction in 83% of the cases. The allergy condition, increased as a result of vaccination, is preserved in all inoculated for one year. This fact is of vast signifiance and is a convincing proof of the high effectiveness of the vaccine.

"After introduction into man of 12.5 million microbes of the dry living vaccine kept for one year, the intracutaneous allergy reaction became positive after 6 days in 50% of the inoculated, after 10 days in 77.3%, and after 15 days, in 100% of the inoculated...

"On the basis of the data studied in the present investigation, it must be concluded that for vaccination of man the most suitable vaccine is from the No. 15 strain.

At the same time, it must be kept in mind that inasmuch as the vaccinal strains are not identical in antigenic and immunogenic respects, the preparation of a polyvalent dry vaccine from several vaccinal strains of tularemia is expedient. This brings forth the necessity of finding new effective strains of the type of no. 15 strain. As a result of the study of a large number of cultures of tularemia bacilli with spontaneously attenuated virulence, we have at the present time found two such strains. One of these, designated No. 10 (with unstudied immunogenic and virulent properties) was obtained in 1944 from the Saratov Institute under the name 'Bulkhta.' This strain is less reactive for guinea pigs and white mice than the No. 15 strain, but the living vaccine from it has high immunogenic properties and protects all guinea pigs (vaccinated with 10,000 microbes) from subsequent infection under the skin (1,000 fatal doses) and through the lungs. White mice, vaccinated with 1,000 microbes of the vaccine from the No. 10 Strain, withstand 1,000 fatal doses of the virulent culture introduced under the skin without the sympton of the illness. These data of experimental study give us the basis to embark on the study of the No. 10 strain in men."

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Q "For the immunoprophylaxis of tularemia (of men), we in the Soviet Union at present (1947) use the antitularemia vaccine from the Ondatra IV and No. 15 (Gayskiy) strains and the dry living antitularemia vaccine of the NIIEG from strains No. 10 and No. 13 (Faybich, Saltykov, and Tamarina)."

Excerpts from the Published Literature on the Development of NIIEG Vaccines
TICO-5954
55-INT-397
Doc. dated 1936-1946

Summary: This report gives date on the production of agent apparently

in test tube lots - very low growth response by our standards. Drying procedures utilizing carbohydrate appear excellent. Immunogenic studies in men, more fully reported in Report II are impressive, not in their detail or interpretations but in practical result. Workers in U.S. should have anticipated this vaccine - Down's Studies with Jap are indicatory.